Restriction analysis of the three plasmids, integration of dCas9_VP64

In charge: Yeast group (Cyril, Loïc, Vincent, Axel, Joseph)

Start date: 30/05 - End date: 30/05 OR Date: 01/06

Aim:

Digest the three plasmids pTPGI_dCas9_VP64, pRPR1_gRNA_handle_RPR1t and plp with restriction enzymes. Then, the electrophoresis will tell if we have the right plasmids.

Schedule :

- Digest the three plasmids with restriction enzymes. Run agarose gel electrophoresis.

Results, discussion and comments:

Step 1: Restriction analysis to check the presence of the three plasmids

Date: 30/05

Duration: 3 hours

Participants:

Axel, Cyril, Loïc

Aim:

Until now, we can't be sure that pTPGI_dCas9_VP64, pRPR1_gRNA_handle_RPR1t and plp are really present in our samples. This step allows to verify by checking the size of different fragments of the plasmid.

Material:

- NEB Buffers (2.1 and 3.1)
- REs (HindIII, XhoI, PstI, BsrBI, EcoRV)
- Plasmid DNA (dCas9 VP-64, gRNA, plp)

Protocol:

Based on NEB protocol (and LISV p62).

Master mix for each plasmid :

- Add 1/10th (1 µL) of the final volume of 10X concentration buffer (NEBuffer 3.1).
- Add water to reach a final total volume of 10 µL.
- Add 1 µg of plasmid DNA.
- Add 1 µg (1 µL) of each restriction enzyme. Restriction enzymes should be last component added to reaction. Keep the restriction enzymes on ice if they leave the freezer.

Samples were loaded according to calc sheet T1 E1 Restriction Analysis

Most enzymes were 100% efficient with NEBuffer 2.1 and 3.1. PstI was 75% efficient with NEBuffer 2.1.

- Mix carefully by tipping the tube or pipetting (no vortexing) followed by a quick spin.
- Incubate your reactions at 37°C for 60 min in heating block.
- Put samples on ice until agarose gel is ready.

Comments:

pTPGI_dCas9_VP64 : HindIII, PstI, XhoI
plp : PstI, HindIII, BsrBI
gRNA : EcoRV, XhoI and BsrBI

If restriction enzymes come from NEB, we have to use the NEB protocol with 10X NEBuffer instead of FastDigest Green Buffer.

Results:

Expected results: See PDF files “Expected Gel.pdf”.
The first lane is for undigested plasmid.

Miniprep of pTGI_dCas9_VP64

Date: 21/07

Participants:

Joseph

Aim:

Isolate the pTGI_dCas9_VP64 plasmids.

Material:

- Overnight cell culture from single colony inoculation
- QIAprep Spin Miniprep Kit Components:
    - P1 = resuspension buffer + RNAse A and LyseBlue
    - P2 = lysis buffer
    - N3 = neutralizing buffer
    - PB = wash buffer
    - Pe = wash buffer
    - EB = elution buffer
    - QIAprep spin column
    - Collection tube
    - 1.5 microfuge tube

Protocol:

protocol from Qiagen Miniprep Kit.

Comments:
We used 3 cultures of 3ml of overnight culture, we pooled them at the begining of the protocole. 2 of the cultures came from one glycerol stock and one from the other.

Results:

The following concentration were obtained :

- 117.1 ng/ul. 260/280 : 1.9 260/230 : 1.68 -> There may be residual guanidine

Storage: In the -20°C freezer.

Prepare for pTPGI_dCas9_VP64 integration

In charge: Yeast group (Cyril, Loïc, Vincent, Axel, Joseph)

Start date: 15/07 - End date: --/--

Aim:

Integrate pTPGI_dCas9_VP64 into yeast genome so that the dCas9_VP64 protein is always expressed by the cells.

Schedule :

- Prepare medium and plates
- Linearize the plasmid
- Integrate in yeast

Step 1: medium preparation

Date: 22/07

Participants: Loic, Joseph

Aim:
Prepare the needed plates and medium.

Material:

Step 2: Test potential single cutters for plasmid linearization

Date: 22/07

Participants: Loic, Joseph

Aim:

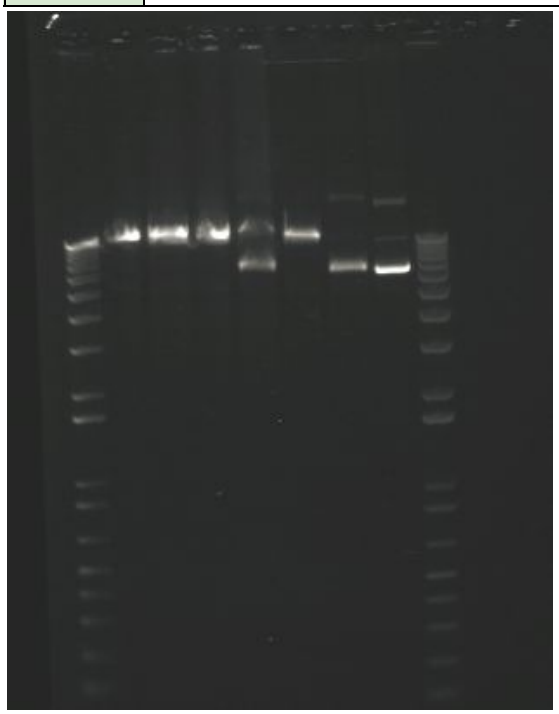
restriction analysis in order to choose the best single cutter.

Material:

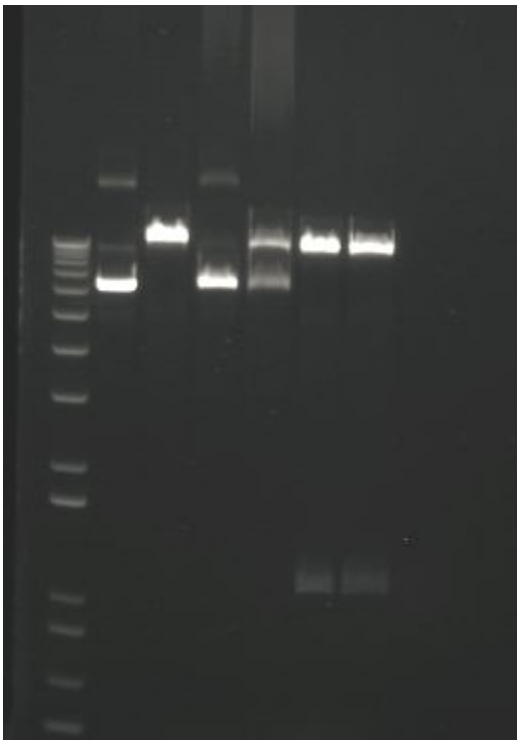
Procedure:

Samples loaded on two gels. Twice too many enzymes but we don't give a shit.

lane	1	2	3	4	5	6	7
enzyme (20 U)	NotI HF	NotI HF	EagI HF	EagI HF	EagI HF	KpnI HF	no
buffer (1 uL)	2.1	cutsmart	2.1	3.1	cutsmart	cutsmart	no
plasmid	pTPGI_dCas9_VP64 (100 ng)						
water	7 uL						8 uL



lane	1	2	3	4	5	6
enzyme (20 U)	no	PciI	Acc651	NaeI	StuI	StuI
buffer	no	3.1	3.1	cutsmart	2.1	cutsmart
plasmid	pTPGI_dCas9_VP64 (100 ng)					
water	8 uL	7 uL				



Results:

Step 3: linearize the plasmid

Date: 22/07

Participants: Loic, Joseph

Aim:
Check linearization.

Material:

Procedure:

Samples were loaded on the gel for verification.

lane	1	2	3
enzyme (40U=2uL)	no	EagI HF	NotI HF
buffer (5 uL)	no	cutsmart	cutsmart
plasmid pTPGI_dCas9_VP64	4 ug	4 ug	4 ug
water	up to 50 uL		

Step 4: transformation protocol

Date: 22/07

Participants: Loic, Joseph

Aim:

integration into genome

Material:

Procedure:

EPFL igem 2014 → protocol of Julie

Result :

transformed yeast on agar plates.
Start of inoculation : 22/07 at 18h.